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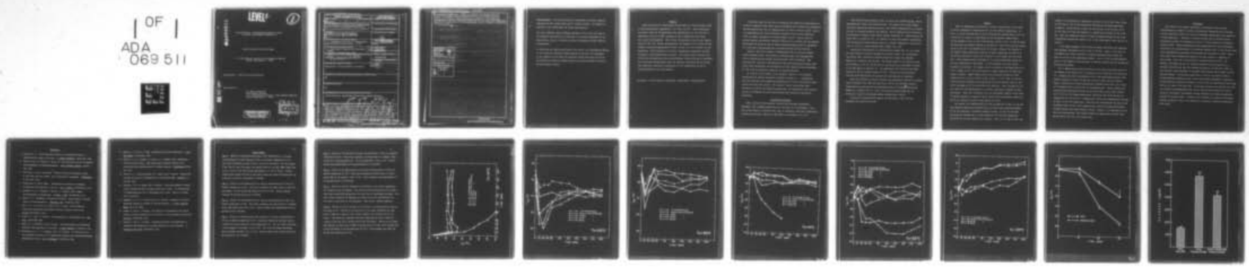

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5-Thio-D-Glucose: Thermoregulatory Effects In Mice At Various Environmental Temperatures

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Running Head: 5-Thio-D-Glucose Hypothermia

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER (6) M 12/79	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) 5-Thio-D-Glucose: Thermoregulatory Effects in Mice at Various Environmental Temperatures		5. TYPE OF REPORT & PERIOD COVERED
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Ralph Francesconi [redacted] Milton Mager (10)		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Army Research Institute of Environmental Medicine, Natick, Massachusetts 01760		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) 6.27.77.A 3E162777A845 94183042
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research and Development Command Ft. Detrick, Frederick, MD 21701		12. REPORT DATE (11) 14 May 1979
		13. NUMBER OF PAGES 11
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Same (14) USARIEM-M-12/79		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution of this document is unlimited. (12) 24p.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

NA

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

5-thio-D-glucose, hypothermia, hyperthermia, thermoregulation

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Adult, male mice were administered various doses of 5-thio-D-glucose (5-TG) at three environmental temperatures: 4°C, 22°C, and 35°C. Both intracerebroventricular (ICV) and intraperitoneal (IP) administration of 5-TG resulted in significant ($p<.05$ to $p<.001$) decreases in rectal temperature (Tre) which were usually dose-dependent. After 30 min the hypothermic effects were significantly ($p<.001$, ICV, 100 µg) exacerbated by cold exposure (4°C vs 22°C) and were likewise intensified significantly ($p<.005$, 45 min, fed vs 18 h fasted) by food deprivation. These decrements in rectal temperature (Tre) were

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accompanied by significant (p<.001) increases in circulating levels of glucose. The present results indicate that 5-TG may be eliciting both central and peripheral cellular glucopenia concomitant with circulatory hyperglycemia; thus, the resultant hypothermia may be arising from competitive inhibition of glycolysis by 5-TG intermediates as well as reduced availability of tissue glucose.

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Acknowledgement: The authors gratefully acknowledge the skilled technical assistance of SP-5 Leonard Sakal and Mr. Stephen O'Connor. We express our gratitude to Ms. Sandra Beach for typing the manuscript.

In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care,' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

Abstract

Adult, male mice were administered various doses of 5-thio-D-glucose (5-TG) at three environmental temperatures: 4°C, 22°C, and 35°C. Both intracerebroventricular (ICV) and intraperitoneal (IP) administration of 5-TG resulted in significant ($p<.05$ to $p<.001$) decreases in rectal temperature (Tre) which were usually dose-dependent. After 30 min the hypothermic effects were significantly ($p<.001$, ICV, 100 μg) exacerbated by cold exposure (4°C vs 22°C) and were likewise intensified significantly ($p<.005$, 45 min, fed vs 18 h fasted) by food deprivation. These decrements in Tre were accompanied by significant ($p<.001$) increases in circulating levels of glucose. The present results indicate that 5-TG may be eliciting both central and peripheral cellular glucopenia concomitant with circulatory hyperglycemia; thus, the resultant hypothermia may be arising from competitive inhibition of glycolysis by 5-TG intermediates as well as reduced availability of tissue glucose.

Key Words: 5-thio-D-glucose, hypothermia, hyperthermia, thermoregulation

For several years we have been investigating the comparative thermoregulatory effects of compounds which affect glucose metabolism both centrally and peripherally, and have demonstrated that the administration of 2-deoxy-D-glucose (2-DG), insulin, or norepinephrine will evoke significant decreases in both rectal temperature (Tre) and oxygen consumption in several species (5,9,14). Results with the glucose analogue and metabolic inhibitor 2-DG led us to conclude that the observed hypothermia was caused by central nervous system glucopenia which is accompanied by a circulatory hyperglycemia and a decreased peripheral heat production.

Recently, Kim and co-workers (7) demonstrated that the glucose analogue, 5-thio-D-glucose (5-TG), was effective in killing cancer cells _in vitro_ when used in combination with hypoxic and hyperthermic (41°C) conditions. They concluded that 5-TG may be interfering with the normal glycolytic process, since earlier Chen and Whistler (2) had demonstrated that phosphorylated intermediates of 5-TG acted as competitive inhibitors of glucose metabolism.

As a result of our interest in the metabolic inhibition and subsequent thermoregulatory effects elicited by analogues of D-glucose, we hypothesized that both centrally and peripherally administered 5-TG would significantly affect normal heat production in mice. We proposed that by quantitating these decrements at several environmental temperatures, the supplementary data would be useful in elucidating the relationship between body temperature and glucose metabolism.

Materials and Methods

Adult, male mice (Swiss-Webster, Charles River Breeding Laboratories, Wilmington, MA), weighing between 28-35 g, were used in all experiments. They were housed singly in stainless steel cages in windowless rooms with automatically controlled fluorescent lights (on, 0600-1800 h), maintained at 22 ± 1°C.

Food (Charles River Laboratory Chow) and water were available ad lib during experimentation except when otherwise noted. The animals were usually weighed and rectal temperatures monitored several times before experimentation to accustom the mice to standard procedures and handling. Rectal temperatures were monitored by inserting a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, OH, Series 702) to a depth of 2 cm. For exposure to other environmental temperatures (Ta) the mice were quickly removed either to a constant temperature, walk-in cold room (4 ± 1°C) or a large stainless steel chamber (35 ± 1°C).

Appropriate concentrations of 5-thio-D-glucose (Sigma Chem. Co., St. Louis, MO) were prepared in sterile, pyrogen-free isotonic saline. For intracerebroventricular (ICV) injections a modified microliter syringe (10) (705-NCH, Hamilton Co., Whittier, CA) was used. The dosage volume for ICV injections was held constant at 0.02 ml, and the site of the injection was determined according to the description of Brittain (1). Intraperitoneal (IP) injections were made aseptically and in a dosage volume of 0.1 ml. Control mice were ordinarily injected equivolumetrically with appropriate concentrations of D-glucose.

Blood was collected by cardiac puncture into heparinized tubes and the plasma rapidly separated (4°C, 4000 rpm) and frozen (-30°C) for subsequent analysis. Plasma glucose concentrations were determined using the Statzyme® (Worthington Diagnostic, Freehold, NJ) kit. This is an enzymatic method based upon the formation of reduced nicotinamide-adenine dinucleotide at 340 nm.

Statistical evaluations were made by the non-paired t test; the null hypothesis was rejected at $p<0.05$.

Results

Figs. 1-3 demonstrate the effects of various concentrations of 5-TG (ICV) on the Tre responses of unrestrained mice at three ambient temperatures. In Fig. 1 it can be observed that by 15 min following the administration of 37.5 μg of 5-TG at 4°C, there is a significant ($p<.02$) hypothermic response which is dose-dependent (e.g. 37.5 μg vs 100 μg dose, 30 min, $p<.01$). At the two lower concentrations the maximal response is observed at 15 and 30 min with Tre increasing by 45 min after the administration of the drug. However, the response to the highest dosage administered (100 μg) is highly significant ($p<.001$) by 15 min and Tre continued to drop until by 90 min 3 of the animals have Tre below 20°C. These animals actually succumbed to the extreme hypothermia before 120 min thus terminating the experiment. At 22°C analogous, but less intense, responses are observed. For example, 30 min following the ICV administration of 5-TG, a dosage level of 50 μg has effected a highly significant ($p<.001$) hypothermic response. Higher dosages elicit more pronounced decreases in body temperature (e.g. 25 μg vs 50 μg, 30 min, $p<.025$). At 35°C (Fig. 3) the dose-response pattern disappears with significant ($p<.05$) decrements noted for only the 50 μg dosage at 15, 90 and 120 min post injection. Thus, decreases in Tre as a result of ICV administration of 5-TG were consistently observed in these experiments. Under cold conditions (4°C) the hypothermia resulted in the death of several animals at a dosage of 100 μg, while under more moderate conditions (22°C) Tre returned to normal levels usually by 60 min.

The results of IP administration of 5-TG are depicted in Figs. 4-6 for the three ambient temperatures studied; while once again dose-response decrements in Tre were observed, a noteworthy anomaly occurred. At each of the three environmental temperatures the lower dosages of 5-TG effected significant elevations in Tre when compared with controls. Thus, at 4°C (Fig. 4) the 5 mg

dosage of 5-TG resulted in a significant increase in Tre ($p<.025$) after 45 min. At 22°C (Fig. 5) the 10 mg concentration effected the following significant increments: 90 and 120 min, $p<.001$; 150 and 240 min, $p<.05$; 210 min, $p<.01$. At 35°C (Fig. 6) 10 mg 5-TG resulted in a significant increment ($p<.02$) at 60 min. Thus, the consistent pattern of these low-dosage increases at all three environmental temperatures appears to be a real physiological effect and not an artefact.

At the higher dosages of 5-TG, there are highly significant and consistent dose-dependent decrements in Tre at both 4° and 22°C. Again, at 4°C (Fig. 4) the highest dose (20 mg) resulted in a marked hypothermia which caused the death of several of the experimental animals. Also, at 35°C the dose-dependent nature of the response breaks down with no apparent differences noted between the 20 mg and 30 mg doses.

Fig. 7 demonstrates clearly the effects of the prior nutritional status of the animals on the intensity of the response to an IP injection of 20 mg 5-TG at 4°C. Animals which were food-deprived for 18 h had significantly greater hypothermic responses at 30 min ($p<.001$) and 45 min ($p<.005$) than fed controls. Figure 8 indicates the intense hyperglycemic responses which occur as a result of IP administration of 20 mg 5-TG in cold-exposed mice. Control animals were injected with physiological saline and sacrificed after 60 min with a minimal Tre of 36.21°C; 5-TG treated animals were sacrificed after 30-60 min with Tre ranging from 27.5°C to 35.6°C. Plasma glucose levels were significantly increased ($p<.001$) in the 5-TG treated mice. When mice were food-deprived and treated with equivalent doses of 5-TG, their plasma glucose levels were again significantly ($p<.001$) greater than saline-treated controls. However, it is important to note also that food-deprived, 5-TG treated animals had significantly ($p<.02$) lower glucose levels than fed, 5-TG treated mice.

Discussion

The results of the present experiments, particularly those relating thermoregulatory responses to various environmental temperatures and nutritional status, demonstrate clearly the role of carbohydrate metabolism in the regulation of body temperature. Initially, it should be noted that we observed several similarities between the mechanism of action of 5-thio-D-glucose and 2-deoxy-D-glucose (2-DG). Several investigators have demonstrated that both intracerebroventricular and intraperitoneal or intravenous administration of 2-DG elicits circulatory hyperglycemia concomitant with intracellular glucopenia (5,9,12). While the present results demonstrate generally the same response, a comparison of data reveals that 5-TG is more effective in generating this action. For example, while Mager et. al. (9) reported increases of plasma glucose up to 120 mg/dl and Muller et. al. (12) observed increases of 70 mg/dl with 2-DG, we have observed a mean increment of 422 mg/dl in fed animals and 270 mg/dl in food-deprived animals. This greater sensitivity to 5-TG is consistently noted in thermoregulatory responses as well, since under similar experimental conditions we have observed equivalent decrements in Tre (2-3°C) for significantly lower doses (100 μg vs 1 mg) of 5-TG than 2-DG. It had been previously hypothesized (15) that the circulatory hyperglycemia which results from the administration of 2-DG is caused by a peripheral catecholamine release effecting an intense glycogenolytic response in storage tissues. Because of the intensity and rapidity of the hyperglycemic response to 5-TG, it is reasonable to postulate a similar mechanism for 5-TG action.

The present results, particularly the profound circulatory hyperglycemia, are consistent with the hypothesis of Freinkel et. al. (5) that the hypothermia results from a depletion of intracellular glucose and certainly not from decrements in circulating levels. Since circulatory hypoglycemia has similarly been related to hypothermia (11,14) especially after insulin administration, it seems apparent that intracellular glucopenia, probably both central at the sights of temperature regulation and peripheral at the sights of heat production, may be responsible for the decreased thermogenesis. This is consistent with our observations with food-deprived mice which showed greater thermoregulatory responses to 5-TG than fed animals concurrent with reduced plasma efflux. The reduced glucose efflux would similarly be consistent with the rapid depletion of both liver and muscle glycogen during even short term food deprivation (3,8).

Our results generally demonstrate significant hypothermia as a result of both ICV and IP administration of 5-TG to mice maintained at ambient temperatures below thermoneutrality. In the 4°C environment the higher doses of 5-TG (100 μg ICV and 20 mg IP) resulted in the hypothermic demise of several of the experimental animals with Tre < 20°C. The rapid and extreme hyperglycemia observed is indicative of a loss of glucose from storage sites probably resulting from rapid glycogenolysis with metabolic inhibition arising from competitive inhibition of glucose metabolism by 5-TG. This is consistent with the apparent importance of carbohydrate metabolism for thermogenesis during acute cold exposure in a variety of species including rats (8), dogs (13), and humans (5). The increased thermoregulatory sensitivity of the animals to 5-TG when compared with 2-DG can thus be attributed to its increased efficacy in stimulating adrenomedullary discharge, increased competitive inhibition of glucose metabolism, or alterations in membrane permeability.

While the present results generally demonstrated hypothermic responses to both ICV and IP administration of 5-TG, it should be noted that at all three environmental temperatures the lower doses of IP administered 5-TG effected consistent and significant hyperthermia. Analogous paradoxical results on thermoregulatory responses have been reported previously for dosage level (4,10), ambient temperature (16,17), and route of administration (10). Hellon (6) has reviewed the extensive differences in thermoregulatory responses noted for various species of experimental animals. Because of the rapid hyperthermia induced by low dose IP administered 5-TG, we hypothesized that lower-dose hyperthermia may be arising from peripheral mechanisms especially since ICV administration consistently effected hypothermic responses. It is possible that in addition to its metabolic inhibitory effects at higher doses, 5-TG could be inhibiting heat loss via peripheral vasoconstrictive mechanisms. It is also feasible that the lower doses of 5-TG are stimulating peripheral glycogenolysis in the absence of sufficient accumulation of phosphorylated 5-TG intermediates to inhibit anaerobic catabolism. Ongoing studies on the thermoregulatory effects of 5-TG on passively and actively heated rats may shed further light on the mechanism of hyperthermia arising from low dose 5-TG administration.

In conclusion, we have found that the administration of 5-TG generally elicits a dose-dependent hypothermia which is most marked at ambient temperatures below thermoneutrality. Further, this hypothermia is concomitant with a highly significant circulatory hyperglycemia, which is accompanied by central and peripheral glucopenia. Also, food deprivation exacerbates the hypothermia of 5-TG administration. Additional studies are necessary to elucidate further the mechanism of its action, and provide insights into the pathways and messengers involved in the central control of peripheral heat production.

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Figure Legend

Fig. 1. Effects of intracerebroventricular (ICV) administration of various concentrations of 5-thio-D-glucose (5-TG) at an ambient temperature of 4°C. Each point represents the mean value of 5 mice for each group. For the control, 25 µg, and 37.5 µg 5-TG groups, the standard error of the mean (SEM) ranged from 0.12 to 1.45; for the 100 µg group SEM ranged up to 2.86 at 90 min. Control animals were treated ICV with an equivalent dosage and volume (50 µg/0.02 ml) of D-glucose in sterile, non-pyrogenic 0.9% NaCl.

Fig. 2. Effects of ICV administration of various concentrations of 5-TG at an ambient temperature of 22°C. Each point represents the mean value of 5 mice for each group. For all groups SEM ranged from 0.03 to 0.64. Control animals received 50 µg D-glucose/0.02 ml 0.9% NaCl.

Fig. 3. Effects of ICV administration of various concentrations of 5-TG at an ambient temperature of 35°C. Each point represents the mean value for 5 animals/group; SEM ranged from 0.09 to 0.50. Control animals were administered 50 µg D-glucose/0.02 ml 0.9% NaCl.

Fig. 4. Effects of intraperitoneal (IP) injection of various concentrations of 5-TG at an ambient temperature of 4°C. Each point represents the mean value of 5 animals per group except the 15 mg dosage where n=4. For the control group and 3 lower dosages of 5-TG SEM = 0.07 to 0.97. For the 20 mg dosage variability again increased and SEM = 0.11 to 2.82. Control animals were treated IP with 10 mg D-glucose/0.1 ml 0.9% NaCl.

Fig. 5. Effects of IP injection of various concentrations of 5-TG at an ambient temperature of 22°C. Each point represents the mean value of 5 animals/ group except the 5 mg dosage where n=4. For all groups SEM = 0.05 to 1.29. Control animals were administered IP 25 mg D-glucose/0.1 ml 0.9% NaCl.

Fig. 6. Effects of IP administration of various concentrations of 5-TG at an ambient temperature of 35°C. Each point represents the mean value of 5 mice/ group; SEM for all groups = 0.06 to 0.48. Control animals were administered IP 10 mg D-glucose/0.1 ml 0.9% NaCl.

Fig. 7. Effects of 5-TG IP (20 mg/0.1 ml 0.9% NaCl) on the rectal temperature of 2 groups of mice (n=4/group). The closed circles represent data for animals which were allowed food *ad lib* until the time when the experiment was begun. The open circles represent the responses of animals which were food-deprived for 18 h prior to the start of the experiment. Mean values ± SEM are depicted.

Fig. 8. Effects of 5-TG IP (20 mg/0.1 ml 0.9% NaCl) on plasma glucose levels of mice kept at 4°C. The bar on the left side of the figure represents the mean value (+ SEM) for a group of fed, control animals (n=5) injected with 0.1 ml saline. The middle bar represents the mean plasma glucose level (+ SEM) for a group of fed mice (n=7) injected with 20 mg 5-TG/animal. The bar on the right side depicts the mean value (+ SEM) of plasma glucose for mice (n=4) treated with 20 mg 5-TG/animal IP and food-deprived for 18 h. Blood samples were taken 30-60 min after injection of 5-TG.

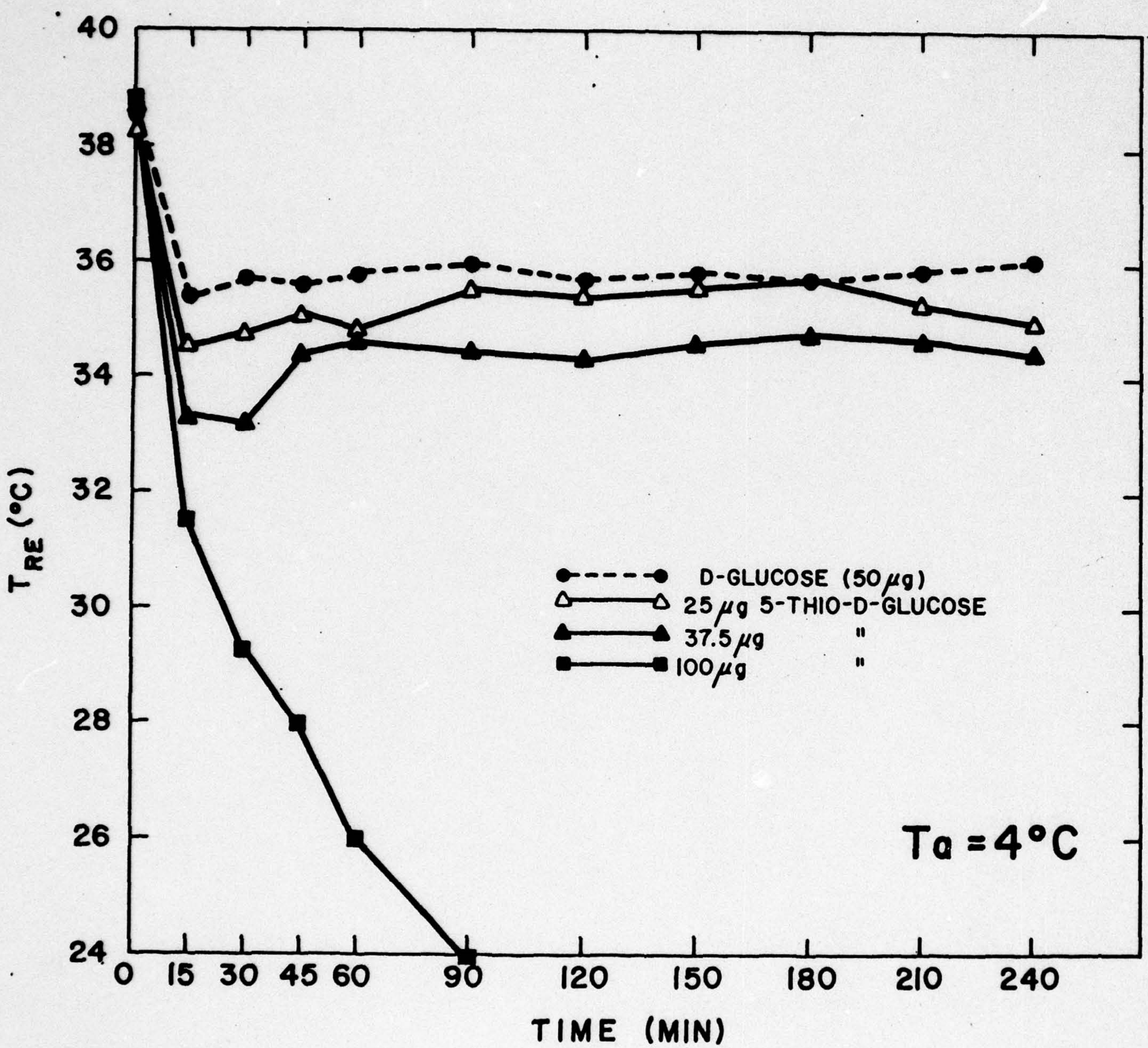

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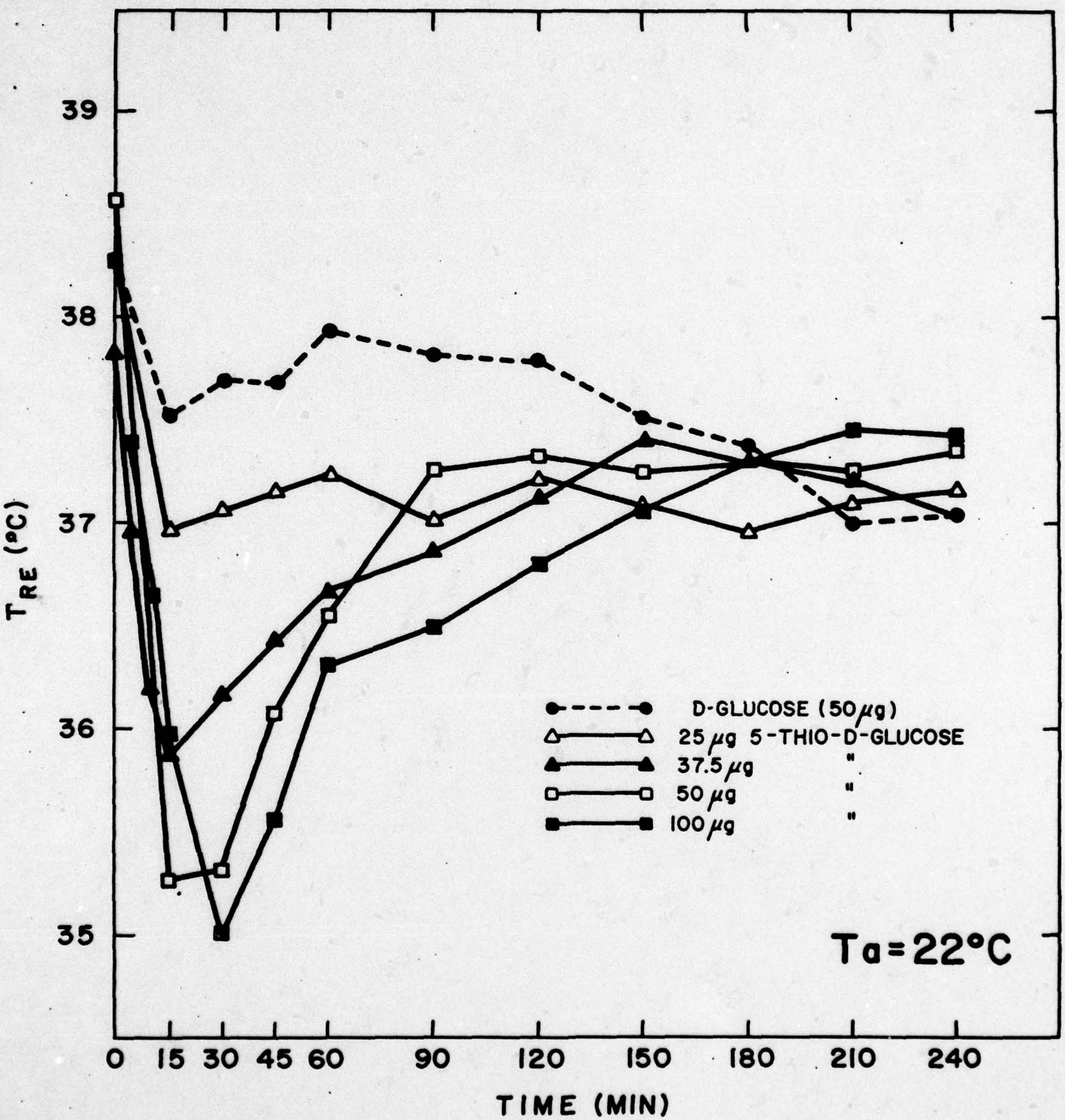
Fig 1



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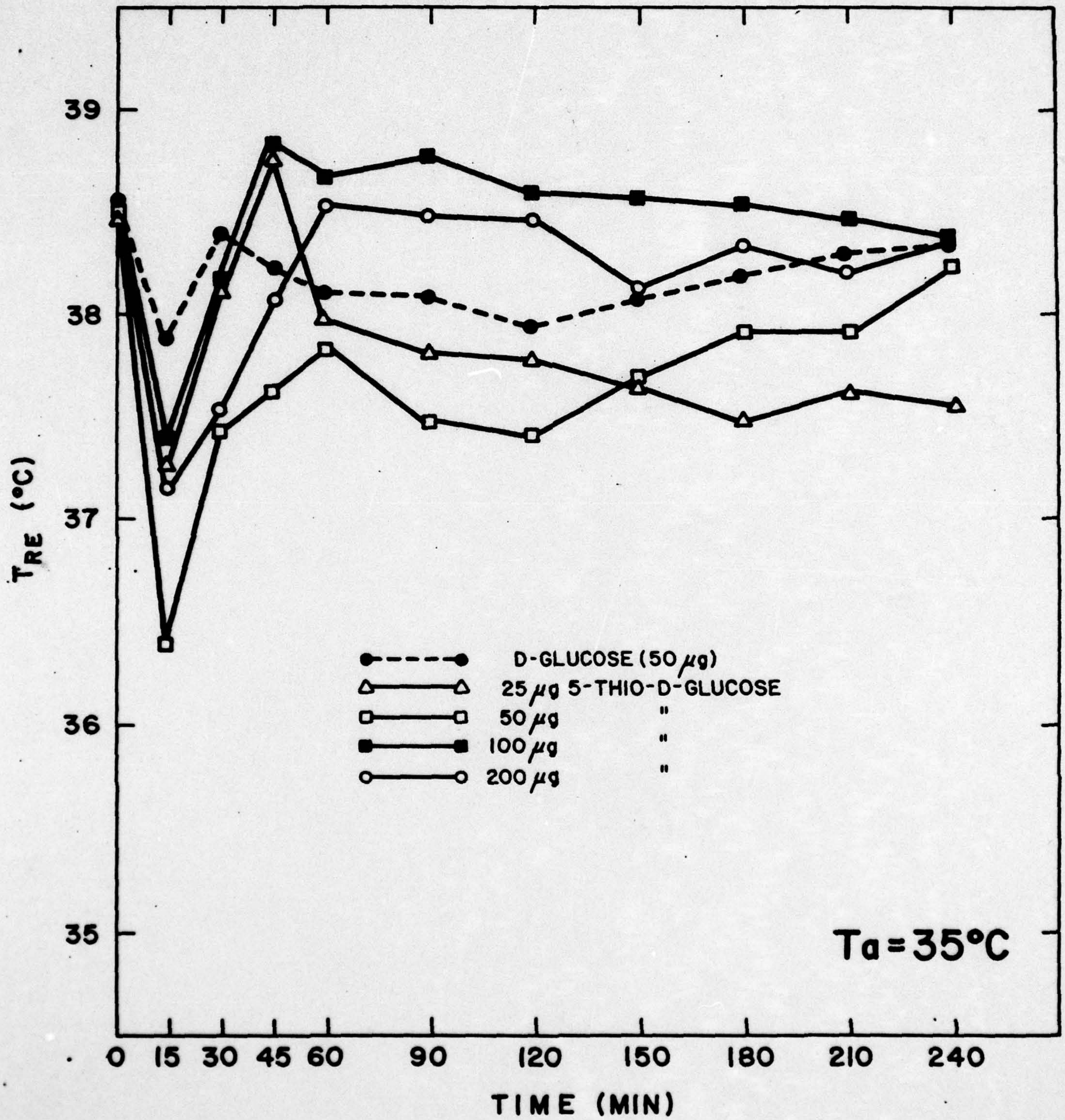
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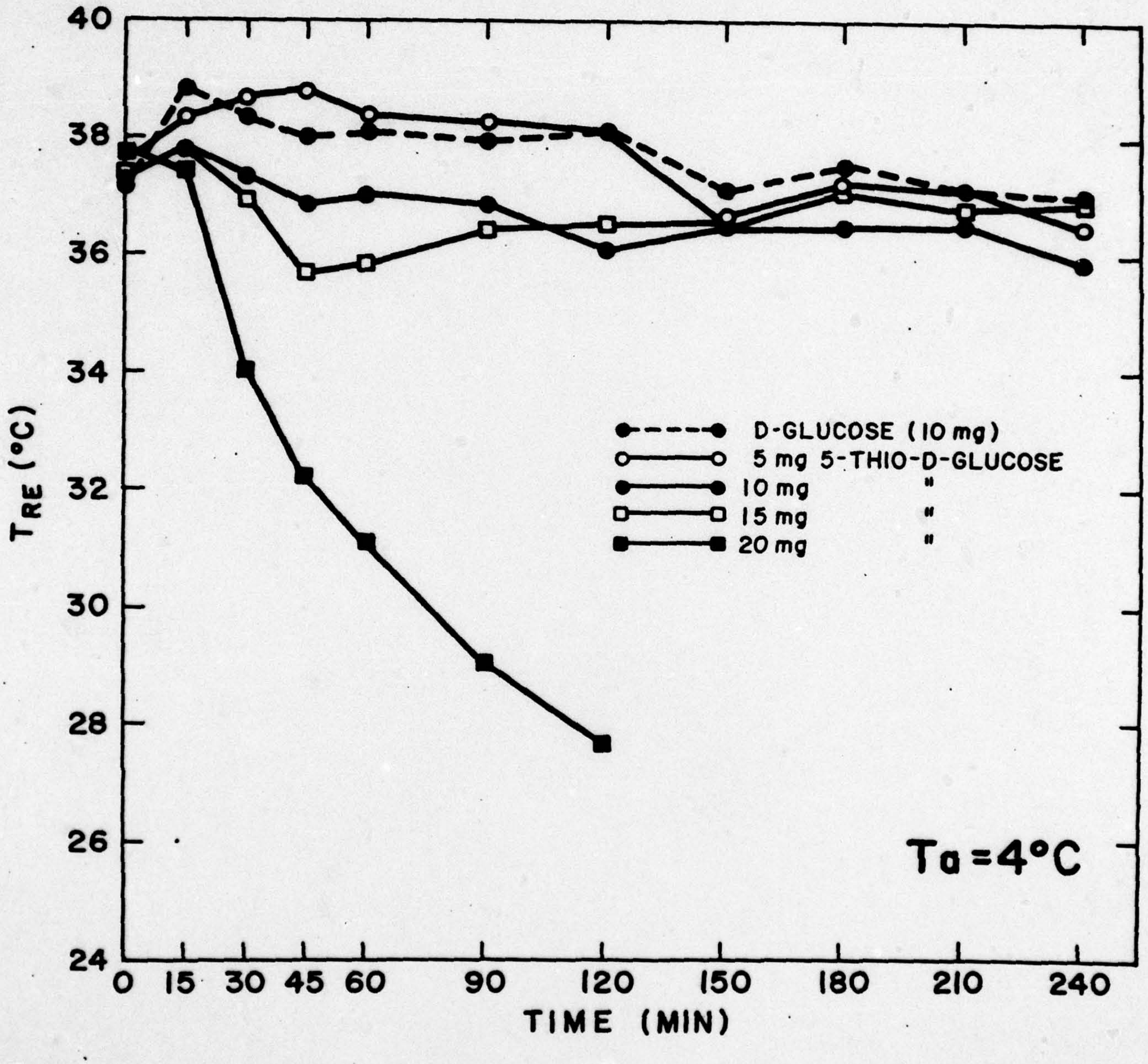

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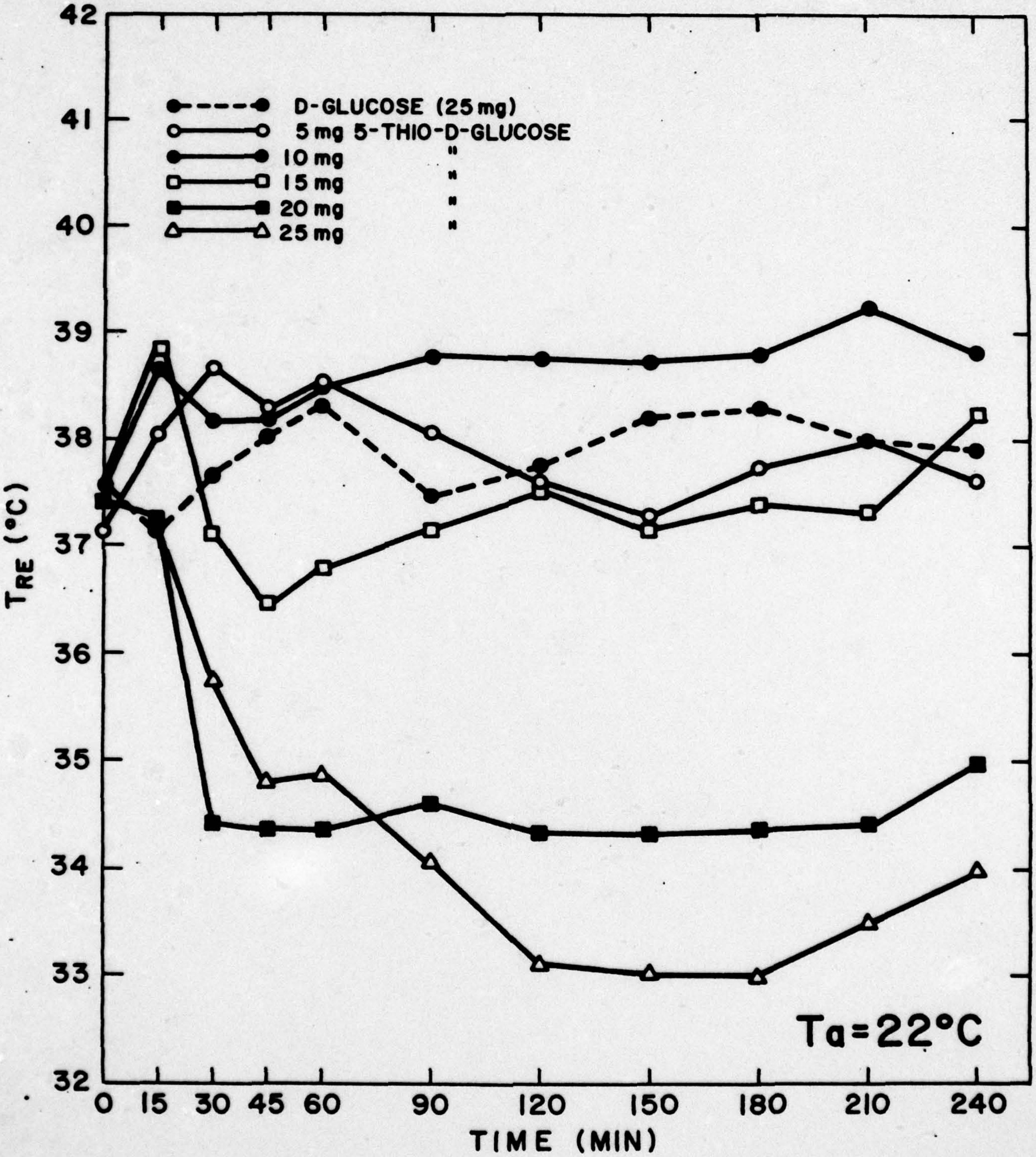
Fig 4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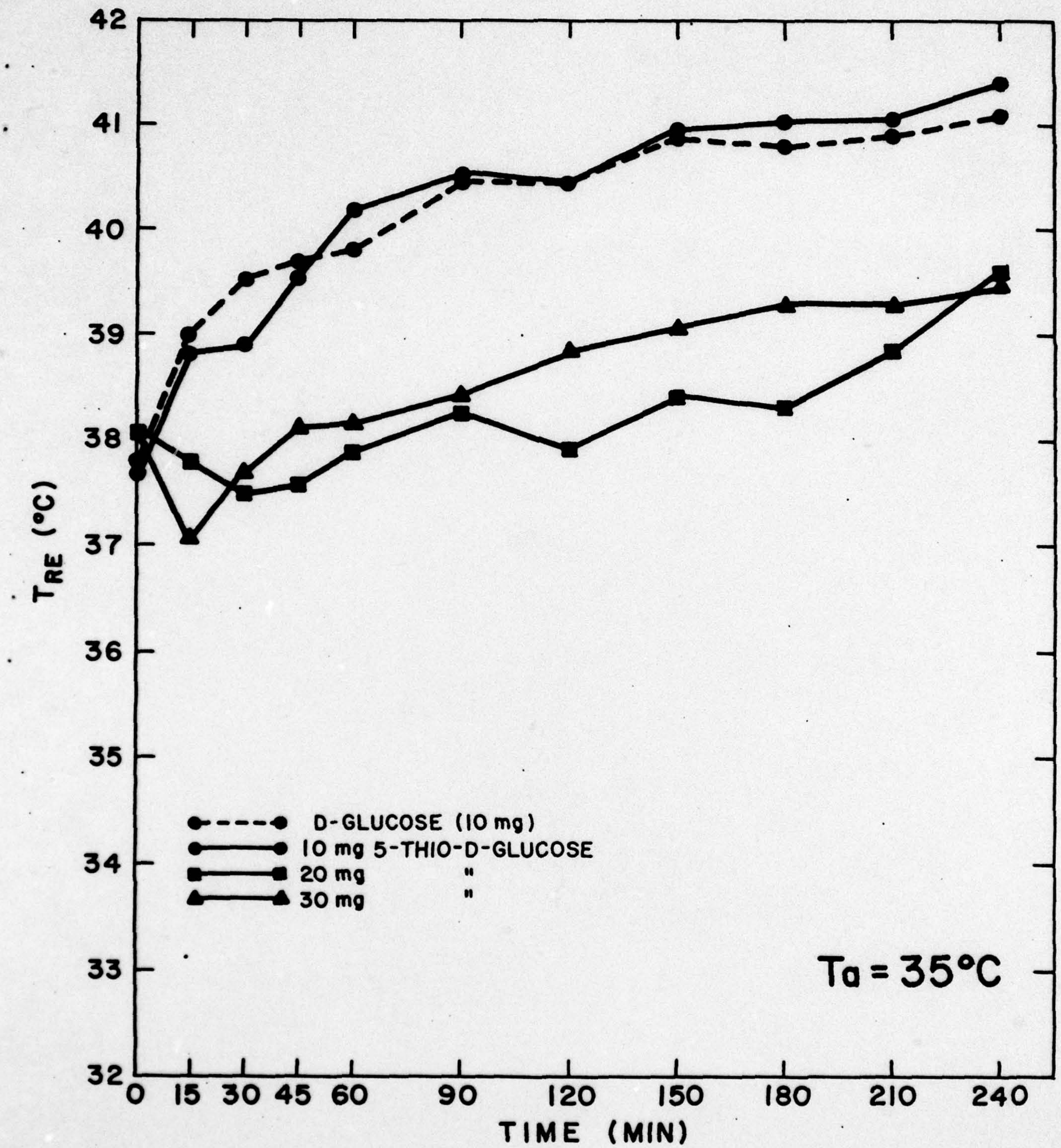

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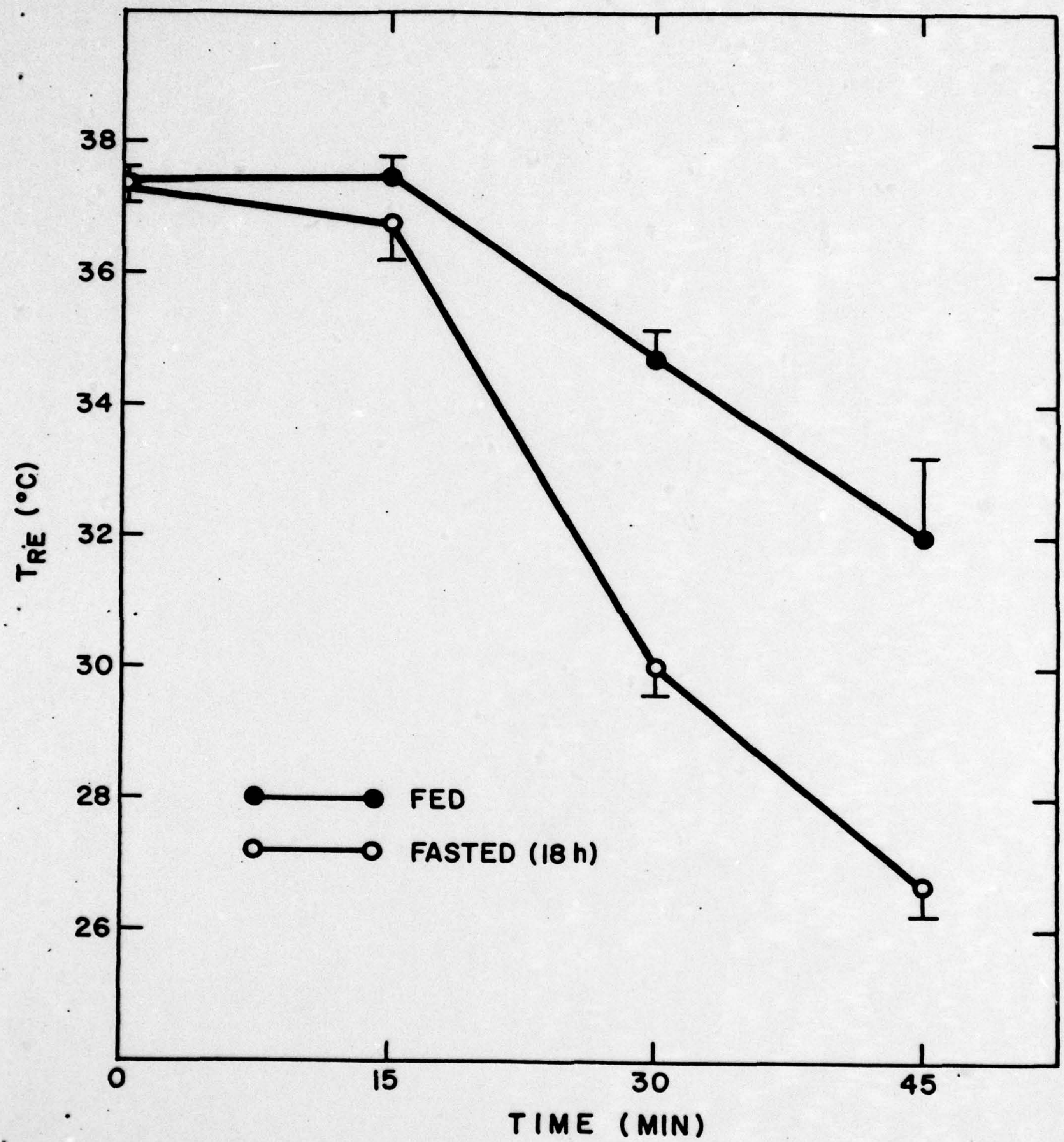
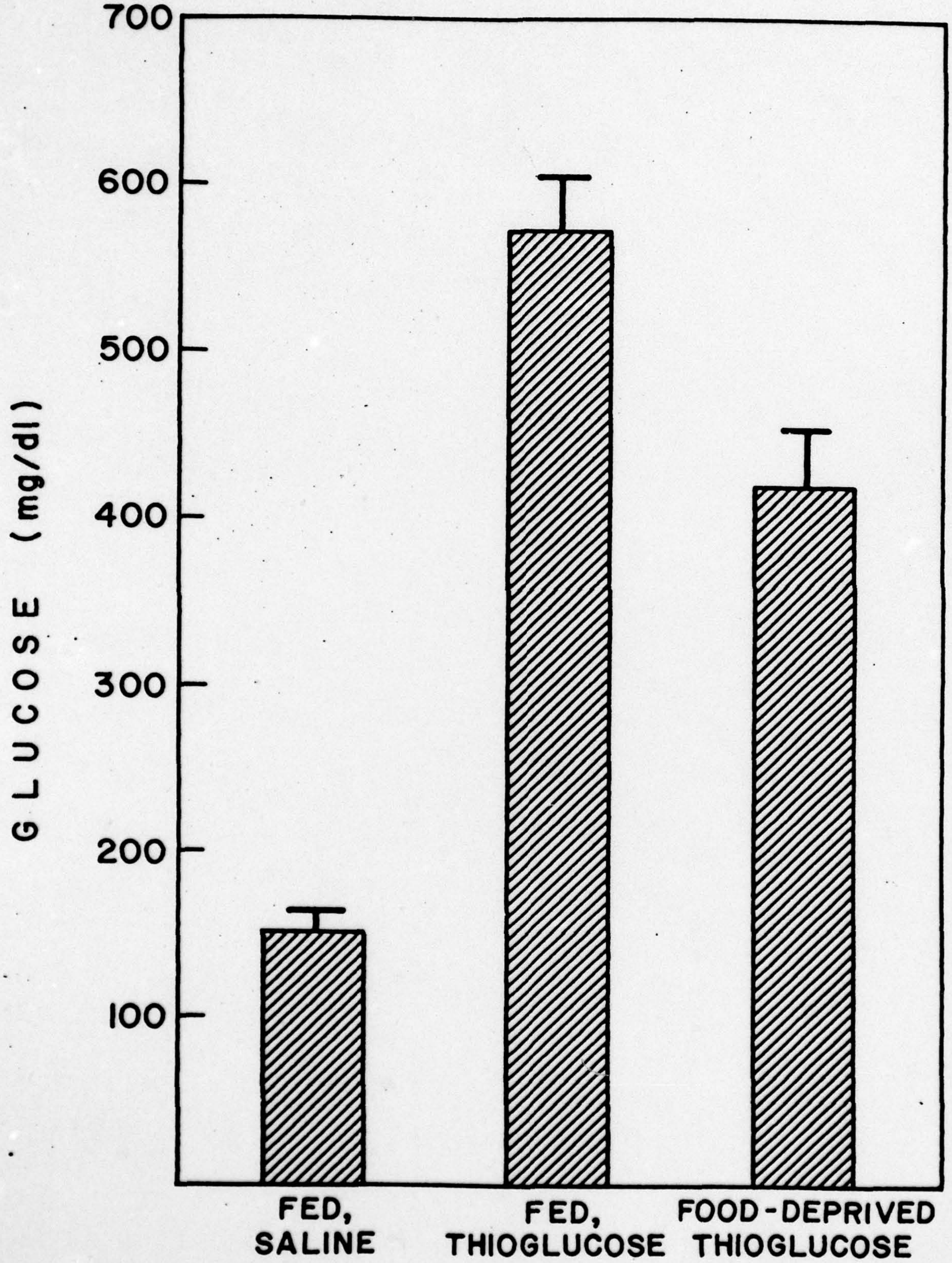


Fig 7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